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Commercial viability of alternative cytoplasmic-nuclear male-sterility systems in pearl millet

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Summary

Commercial viability of three cytoplasmic-nuclear male sterility (CMS) systems (A_4, A_5 and A_v) as potential alternatives to the most widely used A_1 system in pearl millet (*Pennisetum glaucum* (L.) R.Br.) was evaluated in terms of stability of complete male sterility of four isonuclear A-lines ($81A_1$, $81A_4$, $81A_5$ and $81A_v$) and the level and stability of male fertility restoration of their 44 single-cross hybrids. Lines $81A_4$ and $81A_5$ had no pollen shedders (PS), and there were very low frequency of non-PS plants of these A-lines that had a maximum of 1–5% selfed seedset (SSS). In $81A_1$ and $81A_v$, there were, albeit low frequency (<1%) of PS plants, and relatively higher frequency of the non-PS plants in these two lines, the more so in $81A_v$, had 1–5% and even greater SSS. Some hybrids made on each of the three A-lines ($81A_1$, $81A_4$ and $81A_v$) had high and stable male fertility, while others made on the same three A-lines displayed large variation in SSS across the environments, the more so in case of hybrids made on $81A_v$. These results indicate that the A_4 CMS system provides a better alternative to the A_1 CMS system, while the A_v system does not. On the basis of highly stable male sterility and the highest frequency of pollinators behaving as maintainers, the A_5 CMS system appeared to be the best for A-line breeding. The commercial viability of this CMS system in breeding R-lines of grain hybrids, however, still remains to be ascertained as no hybrid on it was fully male fertile in any environment.

Introduction

Discovery of A_1 cytoplasmic-nuclear male-sterility (CMS) system and breeding of a commercially viable male-sterile line (A-line) Tift 23A (Burton, 1958, 1965) is a landmark in pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrid cultivar development. Ever since the development of the first commercial single-cross grain hybrid in 1965 (Athwal, 1965), the Tift $23A_1$ cytoplasm continues to be involved in A-lines of all the grain hybrids. Potential vulnerability of the hybrid seed industry to disease and insect pest epidemics due to cytoplasmic uniformity, as witnessed in case of southern leaf blight (*Bipolaris maydis* (Nisikado) Schoemaker) epidemic on the Texas cytoplasm-based corn hybrids in the United States (Scheifele et al., 1970), has generally been put forth as a strong argument for cytoplasmic diversification of hybrid cultivars. Guided by this concern, efforts have been made to develop alternative CMS sources in pearl millet.

Complete male sterility of A-lines, high levels of male-fertility restoration of their hybrids, stability of these two characteristics across environments, and high frequency of maintainers in a diverse range of breeding materials, are some of the principal positive attributes determining commercial viability of a CMS system. From amongst several alternative CMS sources reported until late 1980s (see Rai & Singh, 1987; Appa Rao et al., 1989: Schertz et al., 1997), most were not evaluated extensively. Amongst those that were evaluated extensively (i.e., A_2 and A_3 systems), none performed any better than the A_1 CMS system with respect to one or more of the positive attributes mentioned above. An A_4 CMS system, developed at Tifton, Georgia, USA, was shown to have more stable male sterility than the A_1 CMS system in

Table 1. Parentage/origin of pearl millet inbred lines used as pollen parents in male fertility restoration study

Pollen parent	Parentage/origin
IPC 382	(B 282 × 3/4 ExB-100-11)-9-2-1
IPC 390	(F_4FC 1498-1-1-3 × J 104)-11-2-1-1
IPC 492	(B 282 × J 804-1-3-9)-7-2-2
IPC 736-3	(ICP 165 × ICP 220)-64
IPC 804-2	(S 10LB-30 × LCSN 1225-6-3-1)-1-2-1-1
IPC 1356-2	(J 1248 × 700112-1)-2-18-1-2-4
IPC 1466	Inbred line from CCS Haryana Agric. Univ., Hisar, India
ICMB 88004	Togo 11-5-2
5054B	Inbred line from Indian Agric. Res. Inst., New Delhi, India
Tift 239D_2B	Inbred line (maintainer of A_2 CMS system) from Tifton, Georgia, USA
L 67B	Inbred line (maintainer of A_3 CMS system) from Punjab Agric. Univ., Ludhiana, India

B282, a breeding line introduced from Malawi; J-series inbreds from Jamnagar Experimental Station of Gujarat Agricultural University, India; F_4FC refers to a breeding line from ICRISAT's Cooperative Program at Kamboinse, Burkina Faso; LCSN refers to a progeny identified at Kamboinse from ICRISAT's Late Composite; 700112, a line from Nigeria breeding program; ICP 165, an inbred from SC 14 (M), which is a mass selected version of Serere Composite developed at Serere Research station in Uganda: S 10LB, an inbred line developed at Punjab Agricultural University, India from a Serere Composite; and ICP 220, an inbred developed from a cross between SD2 and ExB2 (D 1088-1). where ExB refers to Ex-Bornu Composite from Nigeria and SD2 refers to a line derived from Senegal Dwarf Synthetic.

the nuclear genetic background of Tift 23B (Hanna, 1989). A recent study based on isonuclear A-lines in the genetic background of 81B confirmed greater stability of male sterility of A_4 than of the A_1 CMS system (Rai et al., 1996). Another CMS source that appeared to be as stable as the A_1 system in this study was what we designated as A_v (Marchais & Pernes, 1985). These male sterility evaluations, however, were done in a limited number of environments. Rai (1995) identified an A_5 CMS system and suggested, based on a comparison with the A_4 CMS system, that almost every inbred line could be its potential maintainer, implying thereby that the A_5 CMS system might provide the greatest opportunities for both genetic and cytoplasmic diversification. The stability of male-sterility of the A_5 CMS system, however, has not yet been determined.

The objective of this research was to evaluate the stability of male sterility of A_1, A_4, A_5, and A_v CMS systems and fertility restoration behaviour of their hybrids.

Materials and methods

Parental lines and hybrids

The study involved four isonuclear A-lines and 44 single-cross hybrids produced by crossing each A-line with 11 inbred pollen parents. Three isonuclear A-lines (81A_1, 81A_4 and 81 A_v) were developed by more than seven generation of backcrossing of a d_2 dwarf maintainer inbred line (81B) into each of the A_1, A_4 and A_v cytoplasms (Rai et al., 1996). Similarly, the isonuclear A-line 81A_5 was produced by more than seven generation of backcrossing of 81B into the A_5 cytoplasm (Rai, 1995). The 11 pollen parents of diverse parentage, used for producing hybrids, came from several sources, most of these developed at ICRISAT – Patancheru (Table 1). Two of these pollinators (IPC 492 and IPC 804-2) had been selected from amongst seven pollinators used in a previous study for comparing fertility restoration patterns of hybrids made on 81A_4 and 81A_5 (Rai, 1995). Five pollinators (IPC 382=ICMP 501, IPC 390, IPC 736-3=ICMP 85410, IPC 1356-2=ICMP 85303, and IPC 1466=H77/833-2) had been selected from amongst 16 pollinators used in another study for comparing fertility restoration patterns of hybrids made on 81A_1, 81A_2, 81A_3, 81A_4 and 81A_v (Rai et al., 1996). Tift 239D_2B is a dwarf version of Tift 239B (maintainer of the A_2 and restorer of the A_1 CMS system) that was used as one of the 10 pollinators for comparing fertility restoration patterns of hybrids made on Tift 23A_1, Tift 239A_2, Pb 405A_3 and Tift 23A_m=Tift 23A_4 (Hanna, 1989). L 67B is a maintainer of the A_3 CMS system. Since the A_2 and the A_3 CMS systems have been shown to be unstable for their male sterility to the extent that they are of no commercial values (Rai et al.,

Table 2. Mean and maximum temperatures, and relative humidity (RH) during flowering period of pearl millet hybrids (four environments) and A-lines (six environments), Patancheru

Environment[1]	Planting date	Flowering period[2]	Temperature		Relative humidity	
			Mean	Mean max.	0800 h	1400 h
Hybrid evaluation environments						
R95	20 June 95	25 July–21 Aug.	26.5	30.4	91	65
R99	22 June 99	26 July–25 Aug.	24.8	29.1	91	67
DES 96	19 Jan. 96	23 Feb.–25 Mar.	26.9	35.2	70	23
DES 99	29 Jan. 99	4 Mar.–6 April	27.3	36.2	69	21
A-line evaluation environments						
R95	3 July 95	18 Aug.–14 Sept.	26.1	29.9	93	67
R96	24 June 96	4 Aug.–31 Aug.	25.5	28.9	93	74
DES96	30 Jan. 96	18 Mar.–14 April	29.0	36.8	71	23
DES97	6 Feb. 97	1 April–28 April	27.9	34.9	78	28
DLS96	15 Mar. 96	1 May–28 May	32.4	40.4	45	19
DLS97	17 Mar. 97	1 May–28 May	31.2	38.0	64	26

[1] R = Rainy season: DES = Dry early summer; DLS = Dry late summer.
[2] Refers to the period 'a week before the time of the first flowering plot to a week after the time of the last flowering plot' in each environment and each experiment.

1996), neither these A-lines nor any of their hybrids were included in the present study. ICMB 88004 and 5054B are the established maintainers of all the four CMS systems involved in the present study.

Bulk pollen collected from more than 10 panicles of a pollen parent was used for crossing onto each iso-nuclear A-line on the same day. The seed of all the A-lines was produced during the dry season of 1995 by crossing a B-line onto tiller panicles of only those plants of its counterpart A-line which had been confirmed to be male sterile (i.e., no pollen shedding in the main panicle). Similarly, for producing a hybrid, bulk pollen from a pollen parent was crossed onto tiller panicles of only those plants of an A-line, which had been confirmed to be male sterile.

A-line evaluation

An earlier study showed that when A-lines are selfed in a breeding block to determine the extent of selfed seedset (SSS) as a measure of male sterility, mostly 1–5% SSS, though observed in very few plants, do occur and they largely result from contamination with external pollen (Rai et al., 1996). Thus, the A-line nursery in the present study was grown in an isolated field to ensure that no external pollen leads to any contamination of the bagged panicles. The four iso-nuclear A-lines ($81A_1$, $81A_4$, $81A_5$ and $81A_v$) were evaluated for pollen-shedding (PS) plants as well as for SSS in non-PS plants at Patancheru (17°N) during the 1995 and 1996 rainy seasons, and 1996 and 1997 dry seasons (early and late summer), hereafter referred to as environments. This allowed plants to encounter different temperature and humidity regimes during flowering. For instance, the mean maximum temperatures from a week prior to the first flowering plot to a week after the last flowering plot were 29.9 and 28.9 °C and the relative humidity in the afternoon was 67 and 74%, respectively, during the rainy season of 1995 and 1996 (Table 2). For a similar growth period during the late-sown dry season of 1996 and 1997, the mean maximum temperatures were 40.4 and 38.0 °C and RH was 19 and 26%, respectively.

Each A-line was planted on a single ridge of 100 m length, with the number of plants for various A-lines ranging from 355 to 1618. From the beginning until the end of flowering, daily inspections of main panicles for PS plants were carried out in all four A-lines. The number of PS plants were recorded and then they were rogued out as soon as observed. One tiller panicle of those plants that did not shed pollen (i.e., non-PS plants), was selfed at the panicle emergence stage with parchment paper bag to determine selfed seedset (SSS), following the rating scale analogous to the ergot (*Claviceps fusiformis* Loveless) disease (Thakur & Williams, 1980). Individual bagged panicles were classified into four seedset classes: 0, 1–5, 6–20, and 21–50. There were no plants in any A-line with any higher selfed seedset. Considering the cost of SSS evaluation, all of the non-PS plants were not

Table 3. Pollen shedders and selfed seedset distribution in samples of non-shedding (non-PS) plants of four isonuclear A-lines of pearl millet in six environments, Patancheru

A-line	Env.[1]	Pollen sterility		Non-shedding (non-PS) plants				
		Total plants (no.)	Pollen shedders (%)	Total plants (no.)	Percent plants in selfed seedset class			
					0	1–5	6–20	21–50
$81A_1$	R95	519	0.2	518	96.3	3.1	0.0	0.6
	R96	1618	0.6	599	97.8	2.2	0.0	0.0
	DES96	900	0.3	897	93.4	5.2	1.4	0.0
	DES97	1200	0.3	483	95.7	3.9	0.4	0.0
	DLS96	378	0.0	378	99.4	0.3	0.3	0.0
	DLS97	1066	0.0	393	98.7	1.0	0.3	0.0
$81A_4$	R95	478	0.0	478	99.8	0.2	0.0	0.0
	R96	1049	0.0	671	100.0	0.0	0.0	0.0
	DES96	808	0.0	808	97.4	2.6	0.0	0.0
	DES97	1200	0.0	414	100.0	0.0	0.0	0.0
	DLS96	385	0.0	385	100.0	0.0	0.0	0.0
	DLS97	1199	0.0	572	99.5	0.5	0.0	0.0
$81A_5$	R95	459	0.0	459	99.6	0.4	0.0	0.0
	R96	835	0.0	586	100.0	0.0	0.0	0.0
	DES96	718	0.0	718	98.7	1.3	0.0	0.0
	DES97	1167	0.0	575	100.0	0.0	0.0	0.0
	DLS96	366	0.0	366	100.0	0.0	0.0	0.0
	DLS97	1249	0.0	441	100.0	0.0	0.0	0.0
$81A_v$	R95	355	0.3	354	81.4	15.2	2.8	0.6
	R96	690	0.7	449	65.7	30.9	2.8	0.6
	DES96	744	0.6	739	85.8	11.2	3.0	0.0
	DES97	1233	0.3	517	89.7	7.7	2.5	0.0
	DLS96	386	0.8	383	97.9	1.8	0.3	0.0
	DLS97	1116	0.1	462	96.3	2.6	0.6	0.4

[1] R = Rainy season; DES = Dry early summer; DLS = Dry late summer.

necessarily used for this observation, especially when the total number of plants in an A-line were more than a thousand. Also, we did not use for SSS evaluation those very few panicles where the selfing bags had been damaged. Thus, the number of non-PS plants used for SSS evaluation were not always equal to the number of non-PS plants observed during the scoring of pollen shedders.

Hybrid evaluation

Hybrids were grown unreplicated in single-row plots of 4 m length (about 30 plants per plot) during the 1995 and 1999 rainy season, and 1996 and 1999 early-sown dry season at Patancheru, India, hereafter referred to as environments (Table 2). The two rainy season environments differed markedly from the two dry season environments with respect to the mean maximum temperatures and relative humidity. Following the procedure of Rai & Hash (1990). each plot at 75% anthesis was visually scored as: 1 (all plants having shrunken anthers and shedding no pollen), 2 (most of the plants having shrunken anthers and shedding no pollen), 3 (most of the plants having plump anthers and shedding pollen) or 4 (all plants having plump anthers and shedding pollen). None of the hybrids scored as 2 or 3 had pollen-shedding (PS) plant frequencies in intermediate range. PS hybrids were not evaluated for the degree of pollen shed and PS was equated with male fertility. Since the traces of pollen were undetectable to the naked eyes during the pollen shed scoring, SSS was recorded as an additional measure of male fertility. For this, 10 plants in each plot were covered with a parchment paper bag at the beginning of the panicle emergence. Two weeks before maturity, the

Table 4. Pollen-shedding score in hybrids based on isonuclear A-lines of pearl millet in rainy season (1995 and 1999) and dry season (1996 and 1999), Patancheru

Pollinator	Env.	Pollen shedding score[1] in hybrids on							
		$81A_1$		$81A_4$		$81A_5$		$81A_v$	
	Rainy	'95	'99	'95	'99	'95	'99	'95	'99
	Dry	'96	'99	'96	'99	'96	'99	'96	'99
IPC 382	Rainy	4	4	1	1	3	3	4	4
	Dry	4	4	1	1	2	3	4	4
IPC 390	Rainy	4	4	1	1	1	1	2	3
	Dry	4	4	1	1	1	1	1	2
IPC 492	Rainy	4	4	4	4	2	4	4	4
	Dry	4	4	4	4	1	2	4	4
IPC 736-3	Rainy	3	2	4	3	1	1	1	2
	Dry	3	3	4	3	1	1	1	1
IPC 804-2	Rainy	4	3	4	4	1	1	2	2
	Dry	4	3	4	4	1	1	1	2
IPC 1356-2	Rainy	1	2	4	4	1	1	2	4
	Dry	1	2	4	4	1	1	2	4
IPC 1466	Rainy	4	4	1	1	1	1	4	4
	Dry	4	4	1	1	1	1	4	4
ICMB 88004	Rainy	1	1	1	1	1	1	1	1
	Dry	1	1	1	1	1	1	1	1
5054B	Rainy	1	1	1	1	1	1	1	1
	Dry	1	1	1	1	1	1	1	1
Tift $239D_2B$	Rainy	4	4	1	1	1	1	4	4
	Dry	4	4	1	1	1	1	4	4
L 67B	Rainy	4	4	1	1	1	1	4	4
	Dry	4	4	1	1	1	1	4	4

[1]Score 1 = All plants had shrunken anthers and shed no pollen, 2 = Majority of the plants had shrunken anthers and shed no pollen, 3 = Majority of the plants had plump anthers and shed pollen, 4 = All plants had plump anthers and shed pollen.

panicles were scored for degree of SSS following the same procedure as given for A-lines.

Results

Male sterility of isonuclear A-lines

There were no pollen-shedding (PS) plants in $81A_4$ and $81A_5$ in any of the six environments (Table 3). Also, the non-PS plants of these two A-lines had no more than 1–5% selfed seedset (SSS), which occurred in only as few as 0.2–2.6% of the plants of $81A_4$ in three environments and 0.4–1.3% of the plants of $81A_5$ in two environments. In comparison, higher frequency of PS plants and greater degrees of SSS in non-PS plants occurred in $81A_1$ and $81A_v$, the more so in the latter. For instance, 0.2–0.6% PS plants were observed across the rainy and early-sown dry seasons in both years (i.e., four out of six environments) in $81A_1$, while 0.1–0.8% PS plants occurred across all the six environments in $81A_v$. Both of these A-lines registered relatively higher SSS that occurred in all the six environments, with 0.3–5.2% plants of $81A_1$ and 1.8–30.9% plants of $81A_v$ falling in 1–5% SSS class. In 6–20% SSS class also, there were more plants (0.3–3.0%) in $81A_v$ than in $81A_1$ (0.0–1.4%). In both A-lines up to 0.6% plants had even 21–50% SSS, in one environment for $81A_1$ and three environments for $81A_v$.

Fertility restoration of hybrids

Pollen shedding is a qualitative measure of male fertility for which two broad categories of hybrids can be recognized: male-sterile hybrids (score 1 and 2) and male-fertile hybrids (score 3 and 4). Seven hybrids of $81A_1$ were consistently pollen-fertile (scores 3 or 4)

Table 5. Mean selfed seedset in hybrids based on isonuclear A-lines of pearl millet in rainy season (1995 and 1999) and dry season (1996 and 1999), Patancheru

Pollinator	Env.	Mean selfed seedset in hybrids on							
		$81A_1$		$81A_4$		$81A_5$		$81A_v$	
	Rainy	'95	'99	'95	'99	'95	'99	'95	'99
	Dry	'96	'99	'96	'99	'96	'99	'96	'99
IPC 382	Rainy	83	95	2	3	28	48	89	94
	Dry	86	76	<1	2	9	42	58	84
IPC 390	Rainy	94	77	1	2	0	2	40	21
	Dry	73	84	0	0	0	1	1	3
IPC 492	Rainy	90	72	93	75	8	8	86	38
	Dry	85	89	1	27	1	1	45	35
IPC 736-2	Rainy	53	24	91	65	1	2	6	7
	Dry	18	14	38	69	0	<1	1	5
IPC 804-2	Rainy	72	63	93	95	2	2	72	55
	Dry	66	74	95	92	1	2	3	9
IPC 1356-2	Rainy	5	6	79	93	2	1	67	57
	Dry	1	2	88	88	1	0	5	18
IPC 1466	Rainy	91	74	1	2	0	4	81	43
	Dry	60	86	<1	0	0	0	9	22
ICMB 88004	Rainy	1	3	<1	1	0	2	40	29
	Dry	1	1	1	0	1	1	2	2
5054B	Rainy	2	3	15	1	0	0	22	13
	Dry	1	0	1	0	0	0	1	1
Tift $239D_2B$	Rainy	84	53	1	1	2	6	39	25
	Dry	56	24	1	8	1	0	5	4
L 67B	Rainy	92	94	1	2	0	3	94	94
	Dry	95	93	0	1	0	0	91	91

across the rainy and dry seasons (Table 4) and all of these, except the one made with Tift $239D_2B$, also had high (>60%) SSS (Table 5). Three hybrids of this A-line were consistently pollen-sterile and were also consistent for 0–6% SSS. In case of $81A_4$, four hybrids were consistently pollen-fertile, with two of these having high SSS (⩾79%) across the rainy and dry seasons. One of these four hybrids of $81A_4$ involving IPC 492 had very high SSS (75–93%) in the rainy season, but low (1–27%) SSS in the dry season, while the second one involving IPC 736-2 had 65–91% SSS in the rainy season and 38–69% in the dry season. The remaining seven hybrids of $81A_4$ were consistently pollen-sterile, and had mostly 0–3% SSS. One of these hybrids made with 5054B had as much as 15% SSS in the rainy season of 1995. No hybrids of $81A_5$ were found to be consistently good for pollen fertility, and amongst those nine that were consistently pollen-sterile, all had consistently 0–6% SSS. Five hybrids of $81A_v$ were consistently pollen-fertile, but only two of these made with IPC 382 and L 67B registered 58–94% SSS across the seasons. Large variation in SSS across the seasons occurred in the remaining three hybrids, which had 25–86% SSS in the rainy season and 5–45% SSS in the dry season. Similar poor correspondence between pollen fertility scores and SSS occurred in three out of four male-sterile hybrids of $81A_v$ for which SSS ranged from 6–72% in the rainy season, although their low SSS (1–9%) in the dry season was typical of some of the male-sterile hybrids made on $81A_1$ and $81A_4$.

Discussion

Nuclear genetic background of A-lines has been shown to be an important factor influencing the level and stability of male sterility in pearl millet (Rai & Hash, 1990). In this study, use of isonuclear A-lines made it possible to eliminate confounding effect of nuclear genetic background on the expression of male sterility. Male sterile line 81A_1 (A_1 cytoplasm and nuclear genome of 81B) has the highest level of most stable male sterility among the majority of the commercial A_1-system A-lines. Results of this study showed that even in this nuclear genetic background, A_4 and A_5 CMS systems had highest level of stable male sterility as there were no pollen shedding (PS) plants across the environments, very few of the non-PS plants of these A-lines had 1–5% selfed seedset (SSS), and none had >5% SSS. In comparison, 81A_1 had, albeit low frequency of PS plants, and relatively higher frequency of its non-PS plants had 1–5% SSS. In fact, some of the non-PS plants of 81A_1 had even 6–20% SSS across four of the six environments. The male sterility of 81A_v was clearly least stable as it had higher frequency of plants in 1–5% and 6–10% selfed seedset classes than 81A_1.

Most of the male-sterile hybrids in the cytoplasmic backgrounds of A_1, A_4 and A_5 were stable with respect to pollen shedding (score 1) and low SSS (1–5%) across the environments. In the cytoplasmic background of A_v, pollen-sterile hybrids had very low (1–5%) SSS in the dry season, typical of pollen-sterile hybrids, but they had 13–72% SSS in the rainy season. This implies that male sterility of the F_1s made on 81A_v can not be reliably assessed in the dry season, limiting the test to only the rainy season (main season) crop. Thus, based on the stability of male sterility of A-lines as well as that of male-sterile hybrids, the A_4 and A_5 CMS systems provide better alternatives to the A_1 for seed parents breeding, while the A_v system does not.

High frequency of maintainers is another factor that has a direct bearing on seed parents breeding efficiency. Most of the pollinators used in the present study had been selected on the basis of their known male fertility restoration behaviour on A_1, A_4 and A_v CMS systems. Therefore, a comparative assessment of the frequency of maintainers of these three CMS systems in a random sample of inbred lines can not be made. However, hybrids of 81A_5 made with nine out of 11 pollinators (not tested earlier for their fertility restoration of A_5) had complete and stable male sterility across all the four environments, indicating the greatest seed parents breeding efficiency with the A_5 CMS system.

Some of the previous pearl millet studies observed higher frequency of pollen shedders in Tift 23A_1 (A_1 cytoplasm) during the hot dry than in the rainy season and suggested that high temperatures might be involved in sterility breakdown (Balarami Reddy & Reddi, 1970; Thakare, 1977; Saxena & Chaudhary, 1977). These studies, however, neither provided information on the temperature regime nor on the seed source. Our results are contrary to these observations. The mean maximum temperatures during the flowering period in the late-summer season crops were high (40.4 °C in 1996 and 38.0 °C in 1997) with relative humidity (RH) of 45 and 64%, respectively, in the morning hours. At these high temperatures and low RH, there were no pollen shedders in 81A_1, while low frequency of pollen shedders (<1%) in this line were observed in all the other four environments with relatively lower temperatures and higher RH. These results support earlier findings that high temperatures and/or low RH lead to greater expression of male sterility in pearl millet (Rai et al., 1996). In 81A_v, no such relationships were observed between the temperature regimes and pollen shedders. With respect to SSS, however, there was clear indication that high temperatures and low RH in the late-summer dry season crops reduced SSS, especially in 1–5% and 6–10% SSS classes. Further studies are needed to examine if the reduced SSS at high temperatures and low RH was due to greater degree of male sterility, or was it due to longer protogyny (i.e., gap between stigma emergence and anther dehiscence).

There were instances of male fertile hybrids of both A_1 and A_4 CMS systems where the level of fertility and its stability across the seasons was very high. At the same time, there were also instances of fertile hybrids of these both CMS systems where the level of fertility, in terms of SSS, was low, especially in the dry season. These seasonal differences in the level of fertility restoration of hybrids could arise due to different level of pollen production and their viability, or due also to environmentally-induced differences in the length of protogyny. The relative role of these two factors in determining SSS across the seasons warrants further study as it has a direct bearing on restorer parents breeding efficiency of these two CMS systems. Nevertheless, present results do not provide an unequivocal support to previous observations that breeding of stable A_4-restorers would be more efficient than breeding of A_1 restorers (Andrews et al., 1999). With respect to A_v CMS system, there was a clear indication that it would be more difficult to breed highly fertile and stable restorers of this CMS system. Several of pollen-fertile A_v-hybrids had low SSS, especially in the dry season. As far as the assessment of stability of male fertility of hybrids is concerned, it is unlikely that study of hybrids made with a larger set of pollinators will lead to conclusions different from that reached with the hybrids made with 11 diverse pollinators in this study.

Commercial viability of breeding A_5-restorers could not be ascertained in this study as almost all of the pollinators were its maintainers and a few that produced male-fertile hybrids were only partial restorers. Restorer sources conferring high levels of male fertility restoration in A_5-hybrids are rare in the cultivated germplasm and breeding materials (Rai, 1995). Restorer stocks of the A_5 have now, however, been developed that give excellent and stable SSS (>90%) in hybrids (Rai et al., 1999). Backcross transfer of restorer gene(s) from these sources into a diverse range of elite inbred lines and in the A_5 cytoplasmic background, currently at various stages of development, has proved rather simple and straightforward. Fully fertile plants selected in backcross progenies have displayed very high levels of pollen shedding and SSS (Rai, unpublished). Multi-environment evaluation of the derived A_5-restorer lines and their hybrids would provide information necessary to assess relative restorer breeding efficiency with this CMS system.

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